LARVAL DEVELOPMENT OF *CORBICULA FLUMINEA* (MÜLLER) (BIVALVIA: CORBICULACEA): AN APPRAISAL OF ITS HETEROCHRONY

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ABSTRACT

Populations of *Corbicula fluminea* (Müller) in intake bays of Arkansas Nuclear One at Russellville, Arkansas were subjected to a continuing 2½ year study of their gametogenic and ontogenetic processes. Videomicroscopy was especially helpful in working out ontogenetic details, though conventional techniques of microscopic serial sections and scanning electron microscopy (SEM) were also used. In this proto-oogamous species it was found that spermatogenesis is synchronously stimulated by temperature rise in the spring and asynchronously stimulated by temperature decline in the fall. Spermatogenesis, in turn, "times" the process of fertilization and ontogeny. *Corbicula fluminea* seasonally develops many thousands of embryos that characteristically differentiate into blastulae, gastrulae, trochophores, veligers, pediveligers and early and late, straight-hinged juveniles. The fall reproductive pulse lasts about 14 days longer than the spring pulse and fall is the only time that evidence of self-fertilization has been gathered. Neither the trochophores nor the veligers appear to be well adapted for a freshwater, planktonic habit. Late pediveligers and early to late juveniles are the stages of development usually shed from the parent clam. Once released from the marsupial gill into the lotic environment, the straight-hinged juvenile grows into an umbonal juvenile at about 500 μm. About three months were required for development of a straight-hinged juvenile into an umbonal juvenile, in laboratory culture. When the shell valves of the umbonal juvenile attain a length of about 1 mm, a byssus is developed. Chronicity of ontogeny is compared with that of certain marine bivalves and with indigenous freshwater corbiculacean relatives of *Corbicula fluminea*, the pill clams and fingernail clams (Pisidiidae). We argue that heterochrony, in the phyletic, evolutionary sense in which it was used by De Beer, very likely accounts for much of the current "success" of *Corbicula fluminea* in the United States.

During a study of the biota of the Arkansas River in Arkansas, in 1974-75, it was found that juvenile *Corbicula fluminea* were the most abundant and widely distributed organisms, by far, in the benthic communities of the 672-kilometers-long study reach (Kraemer, 1976). Ponar grab samples obtained in the study contained thousands of tiny (1-4mm long) clams. Many of the clams were removed with their byssal thread still intact and adhering to sand grains from the substratum (Kraemer, 1979). Sinclair and Isom (1963) had found the veligers of the clams to be "short-term planktotrophic, non-swimming" larvae, which were discharged from the gravid clams into the surrounding water. The only developmental stage which appeared in our samples, however, was the well-differentiated juvenile.

Another finding which emerged from the 1974-75 study was that upstream populations of juvenile *C. fluminea* showed some evidence of recruiting to the downstream populations in successive seasonal sampling series. Though the point was not emphasized at the time, some of the figures (Kraemer, 1979, Figs. 4,5,6) provide the basis for such an interpretation. It seemed that most of the young shed into the environment were juveniles. The juveniles differentiated a byssal thread following their release into the stream and tended to remain close by. Over a period of several months, however, juvenile *C. fluminea* could be transported downstream, perhaps along with sand grains to which their byssus attached, to populate the downstream benthos.

A "clam clog" of the service water system of Arkansas Nuclear One, located on the Arkansas River near Russellville, Arkansas, forced the costly shutdown of the facility in the fall of 1980. The present study grew out of the urgent need for a clear understanding of the details of the reproduction and developmental cycle of *C. fluminea* in the intake bays at Arkansas Nuclear One. From the spring of 1982 to the fall of 1984, populations of *C. fluminea* were subjected to continuing analysis of their gametogenic and ontogenetic

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processes.

Earlier studies on the freshwater corbiculacean relatives of *C. fluminea*, the Pisdiidae (pill clams and fingernail clams), such as those by Heard (1977) and Mackie, *et al.* (1974a,b) afforded a basis of comparison with emerging details on reproduction and development in *C. fluminea*. Kraemer and Lott, (1977), Kraemer (1978, 1979a, 1979b, 1984, in press) and Kraemer et al. (in press) had worked out a series of details, including the fact that *C. fluminea*, unlike the Pisidiidae, is proto-oogamous in its development. Morton (1982) reviewed characteristics of reproduction in *C.* cf. *fluminalis* from the Pearl River near Canton, China, noting that *C.* cf. *fluminalis* (ibid, p. 18) shows ". . . a general trend towards protogynous hermaphroditism" and that *C. fluminea* (in Hong Kong) is ". . . a protandric hermaphrodite." Some details of reproduction and development reported Sinclair and Isom (1963), Aldridge and McMahon (1978) by Eng (1979), and Kraemer (1978, 1979) were evaluated by McMahon (1983) in a comprehensive review of work to date on the ecology of *C. fluminea*.

The present study includes sufficient data to provide the basis for a clear understanding of (1) the role of gametogenesis in the life cycle of *C. fluminea*; (2) the functions of the spring and fall reproductive periods; and (3) many details of embryogenesis. The timing, appearance and behavior of the characteristic embryonic stages of *C. fluminea* presented here, support our hypothesis that the present "success" of *C. fluminea* can be accounted for largely by the heterochronicity of developmental events in its life cycle. Heterochrony is a newly revived idea, rather than a new idea in Biology. Chief among modern explicators of the concept of heterochrony is Stephen Jay Gould. We invite the reader to consider the historical usage of heterochrony as reviewed by Gould (1977), p. 402):

"HETEROCHRONY 1. According to Haeckel, displacement in time of ontogenetic appearance and development of one organ with respect to another, causing a disruption of the true repetition of phylogeny in ontogeny. The embryonic heart of vertebrates, for example, now appears far earlier in ontogeny than its time of phylogenetic development would warrant.

2. Cope used the same definition as Haeckel, but viewed heterochrony as support for the biogenetic law. Recapitulation must be defined organ by organ, not in terms of the whole body. The heart may be far more strongly accelerated than other organs, but it is still accelerated, and acceleration is the mechanism of recapitulation.

3. De Beer defines heterochrony as phyletic change in the onset or timing of development, so that the appearance or rate of development of a feature in a descendant ontogeny is either accelerated or retarded relative to the appearance or rate of development of the same feature in an ancestor's ontogeny."

The reader will note that all of the above definitions concern the matter of *timing* of ontogenetic events and the reasoning that *change in timing* can produce evolutionary change in populations of organisms over generations. The techniques of videomicroscopy and SEM today permit careful monitoring of minute developmental events in the dynamic ecology of molluscan embryos. It is now possible, we think, to extend, amplify and refine the concept of heterochrony, and to advance it as an explanatory principle, for example, for the present ecological position of *C. fluminea* in the U.S. In what follows, the reader is asked to note both the timing and the sequence of developmental events in *C. fluminea*. The reader is also asked to recall that *C. fluminea* characteristically achieves huge biomass in situations of "ecological crunch" (Wiens, 1977), in this instance in U.S. river systems which have been greatly altered by dredging, damming, channelization, and heated effluents, etc.

MATERIALS AND METHODS

From the spring of 1982 through the summer of 1984, specimens of *C. fluminea* were taken from the intake bays at Arkansas Nuclear One near Russellville, Arkansas and shipped to our laboratory in Fayetteville. This was done at monthly intervals in December, January and February, biweekly during early spring and late fall, and twice a week to daily during peak reproductive periods in spring and fall. During this period we periodically collected *C. fluminea* from populations in the White River in Washington County, Arkansas and from the Llano River in Llano County, Texas, for purposes of comparison with the Arkansas River clams.

From May, 1982 to May, 1983, careful dissections of hundreds of clams were carried out in order to obtain an understanding of many aspects of gametogenesis and embryogenesis. Early in the study we realized that ANO personnel were finding embryos in the gills of *C. fluminea* at Russellville often when we were not able to find them in the clams they had sent to Fayetteville. Subsequent checking revealed that the clams, shipped in containers of river water, prematurely shed their embryos during transit. This occurred despite the fact that the shipping distance was less than 160 km, and the clams were cooled during shipment. We found that shipping the clams simply wrapped in moist toweling and cooled, lessened the likelihood of their losing embryos during the journey.

By May of 1984 protocols for evaluation of gametogenic and embryogenic events had been developed and standardized. The protocols provided a consistent method by which details of the developmental process in *C. fluminea* could be worked out. They are purposively quite different from study procedures prescribed by Britton and Morton (1982). Until examined, (usually within 48 hours of shipment) the clams were kept in the cool, moist toweling in which they had been shipped, to prevent shedding of embryos from the marsupial gills. Ten clams from each shipment were systematically treated as follows.

(1) Great care was taken to preserve the integrity of the mantle and the visceral mass during dissection. Forcing the valves slightly apart with a scalpel and holding them thus with one's thumb, an iridectomy scissors was used to cut through the siphons and the posterior adductor muscle (*between* the mantle lobes), and then to cut between the mantle lobes through the anterior adductor muscle. The left mantle lobe was then carefully separated from the left shell valve

and lowered onto the visceral mass. The left shell valve was then removed.

(2) The left mantle lobe was next gently pulled back to expose the gills and the visceral mass. Gills were examined *in situ* with a dissecting microscope for the presence of embryos or larvae. Gills were not removed at this time but were simply folded back to expose the surface of the visceral mass. Using an iridectomy scissors, two incisions were then made. One incision was made parallel and near to the base of the left inner gill. The second incision was cut along the anterior margin of the visceral mass. A jeweler's forceps was used to grip the covering epithelium of the anterodorsal aspect of the visceral mass, near the digestive glands. The epithelium was carefully pulled back, exposing any peripherally located oogenic and spermatogenic follicles.

(3) When present, spermatogenic follicles were located and counted. We found that spermatogenic follicles may be reliably detected when they appear, as a few, whitish, finely granular masses just under the translucent membrane of the visceral mass. Each follicle mass measures about .25mm to .5mm in diameter (Kraemer and Swanson, in preparation).

(4) Spermatogenic follicles were removed from several different locations on the visceral mass. Smears of the tissue were made and examined with an AO 110 Phase-Star compound microscope. Stages of spermatogenesis were identified and characterized as: (a) "Bead." Follicles with few or no mature sperm present. Follicles appear finely granular or bead-like; (b) "SF sperm." No distinctive appearance of the follicle, but many sperm in various developmental stages present; (c) "Ball stage." Follicles typically packed with hundreds of spheres of mature sperm. Kinds and relative proportions of sperm present (round-headed, wide-headed, slender-headed) were determined by means of criteria established earlier (Kraemer and Swanson, in preparation).

(5) Smears were then made of oogenic tissue to determine appearance and size of the oocytes present. In this and all of the foregoing dissections and smear preparations, great care was taken to prevent contamination of the visceral mass by embryos from the marsupial gills.

(6) The visceral mass itself was examined for the presence of embryos, since they had been observed repeatedly by Kraemer (1978) within the oogenic follicles within the visceral mass, in serially sectioned clams. During the course of the current study, several observations of living, early embryos were made from follicular tissues of the visceral mass. Implications of these findings for self-fertilization of *Corbicula fluminea* are discussed further below.

(7) Following detailed dissection of the visceral mass, all four gills were examined. All gills containing embryos (usually just the inner gills) were removed by cutting along their bases with an iridectomy scissors. The gills were placed on a slide in a few drops of conditioned water (i.e. water in which the clams were maintained in the laboratory). Embryos were freed from the marsupial gills by gently teasing the gill tissues apart. The subsequent, mixed sample of embryos was scrutinized to determine kinds of embryonic stages present. All embryos from each gill were counted and categorized if less than 100 were present in each gill, as follows: (a) no embryos present; (b) cleavage, blastula; (c) gastrula; (d) trochophore; (e) veliger; (f) pediveliger; (g) early, straight-hinged juvenile; (h) late, straight-hinged juvenile. If embryos were more numerous, a representative subsample would be similarly counted and categorized. Sometimes the procedure was carried out several times for a clam, when its marsupial gills were charged with thousands of embryos. This was done to ensure adequate representation of the embryonic stages present. Subsample counts from each gill were averaged to determine relative frequency of each developmental stage.

(8) In addition to the foregoing steps routinely carried out on 10 clams per sample, additional clams were examined from each sample in order to obtain further information on developmental sequences, spermatogenesis, follicular development, behavior, state of the different developing tissues and organs, etc.

(9) Many other clams in each sample were used for the purpose of refining our observational techniques with Scanning Electron Micrography, videomicroscopy, phase microscopy, photomicrography and histological techniques.

At the beginning of the study and at intervals throughout the study, careful reference was made to a large series of microscopic serial sections of *C. fluminea* which had been prepared earlier (Kraemer, 1978; Kraemer and Lott, 1977), of a number of clams from the Buffalo River in Arkansas, over the space of 1½ years (1975-1977). During the present study, additional serial sections were prepared of gravid gills of *C. fluminea* containing mostly juvenile clams. All sections were stained with an aniline blue variation of Mallory's Triple Stain (Scmitz, 1967).

A Wild steromicroscope was used in conjunction with a 35 mm Wild MKa 1 camera to visualize and photograph living embryos during the early part of the study. Later a compound AO Microstar microscope fitted with a Panasonic, PK-972 Color Videocamera, and attached to a Panasonic VHS Recorder was used to produce images of living tissues, gametes and embryos on a 19-inch TV monitor. This apparatus provided high-resolution, magnified images of the living embryos and allowed detailed analysis of embryonic behavior as well as of tissue/organ development of the semi-transparent embryos.

Preparation of tissues for SEM involved fixation in 2.0% glutaraldehyde and subsequent processing through cold phosphate buffer solutions and a dehydration series of ethanols. Following critical-point drying with liquid CO_2, the tissues were mounted on studs with silver adhesive solution and coated with 15 nm of gold, using a Polaron SEM Coating Unit, E500. Alternatively, the tissues, following dehydration, were enclosed in small (1 cm^2) packages of Parafilm, immersed in liquid nitrogen, then removed and freeze-cracked by wielding a hammer against a razor blade held on the tissue. These tissues were then mounted, cracked surface up, on the studs before coating. All tissues were then viewed with an ISI-60 Scanning Electron Microscope at 30 Kv and a working distance of 15 nm.

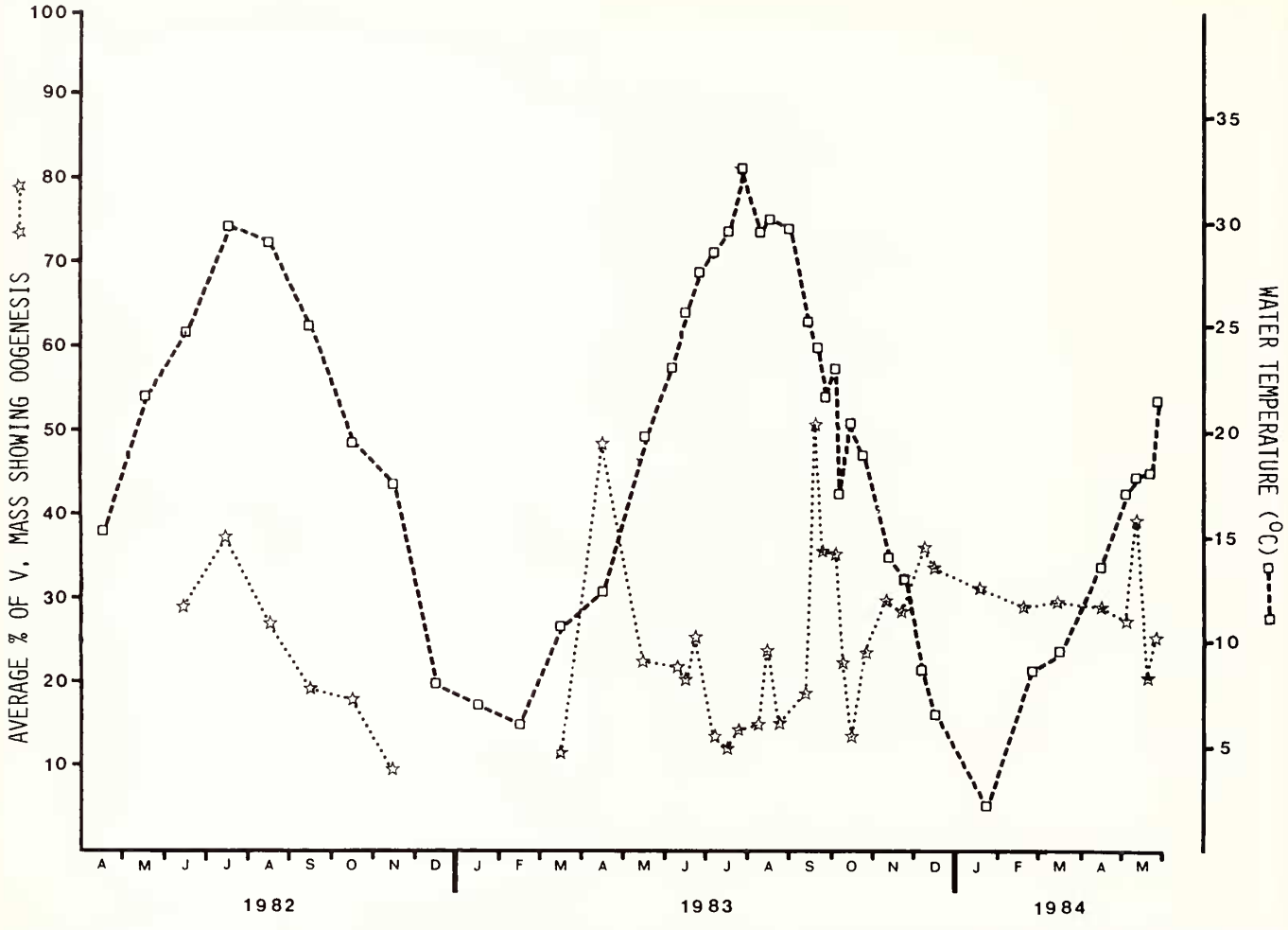

Fig. 1. Average extent of oogenesis in the visceral mass of *C. fluminea* in relation to water temperature in ANO intake bays (Arkansas River) near Russellville, Arkansas.

RESULTS

GAMETOGENIC-ENVIRONMENTAL TIMING

Earlier it was found through microscopic serial section study (Kraemer, 1978) that: (1) Ontogenetically, *C. fluminea* is proto-oogamous, as gametogenesis is initiated when oogenic follicles begin to form in association with the basement membranes of the mucosa of the digestive glands or gut wall. (2) In contrast to the sequence in many bivalves, including the freshwater corbiculacean relatives of *C. fluminea* (family Pisidiidae), the pill clams and fingernail clams, oogenesis seasonally precedes spermatogenesis. (3) It is only when oogenesis is well underway and the oogenic follicles have branched and ramified through the visceral mass, that spermatogenic follicles appear, peripheral to the oogenic follicles.

In the course of this study, which involved careful dissections of fresh tissues of approximately 2000 specimens, the above observations were confirmed and amplified. We now know that in the ontogeny of *C. fluminea*, oogenesis is not only the first form of gametogenesis to occur, but that once begun, it continues throughout almost the entire year in the mature clam. In all months, clams were examined and were found to have all three size classes of ova present in their oogenic follicles. That is, the oogenic follicles contained oocytes measuring $< 90\ \mu m$, $\geq 90\ \mu m$, $\geq 140\ \mu m$. There were far fewer of the small-sized oocytes in the oogenic follicles during January, February and March. In April of 1983 and in early May of 1984, there was a marked increase in oogenic follicle development one to two weeks before the appearance of embryos in the gills. In both 1983 and 1984, however, the *onset* of spermatogenic follicle development *preceded* the spurt in oogenic follicle development by 1-4 weeks (Figs. 1,2). Embryogenesis (Fig. 3) followed both.

We now know that *spermatogenesis is definitely a seasonal phenomenon.* We have accumulated evidence indicating rise of spring water temperature to 10°C or more for 7 to 10 days initiated spermatogenesis in 30%, 28% and 42% of the clams in 1982, 1983 and 1984, respectively. Synchronous development of spermatogenic follicles was found in virtually all clams exhibiting spermatogenesis. After

spermatogenic follicle development and concomitant spermatogenesis have continued for two to two and a half weeks, and spheres of mature sperm are regularly seen in fresh dissections, spermatogenesis diminishes and the follicles atrophy. After a period of rising water temperature to 17-19°C for 7-10 days, embryos appear in the gills (Fig. 3). During early summer months there is more variability than there is in the synchronous development of spermatogenic follicles which accompanies water temperature rise in the spring. However, there may be three or four wave-like recurrences of series of developmental stages of *C. fluminea* embryos. It is as though the temperature-induced, spermatogenic spring "pulse" brought about a reverberating series of developmental sequelae in the adult clam. Late in July, apparently in response to sustained high water temperature (29°C or higher) the reproductive-developmental sequence is interrupted.

The fall reproductive period was initiated in midsummer when the water temperature fell below 29°C. Evidently because the water temperature fluctuated much more in summer than in the spring, *and* because of metabolic demands already put on energy stores of the clams during spring and summer, onset of spermatogenesis during this period was not synchronous across the population as it had been in the spring. As a consequence of asynchronous spermatogenesis, (more variability of spermatogenic follicle stages present) ensuing embryogenesis was also less synchronozed. In three fall seasons encompassed by this study, the fall reproductive period lasted longer (by an average of 14 days) than the spring pulse (Fig. 3). In both fall and spring several cleavage-to-late-juvenile sequences were seen.

There is some evidence that the fall reproductive pulse is the strongest one: (1) Only in the fall did we make occasional observations of clams with fully gravid inner gills *and* with several water tubes of one or both of the outer gills containing embryos. (2) Our observations of evident self-fertilization were made on clams collected in the fall. Only in the fall did earlier serial section studies (Kraemer, 1978) reveal the presence of *intrafollicular embryos* in the visceral mass. Only in the fall were embryos occasionally seen in fresh

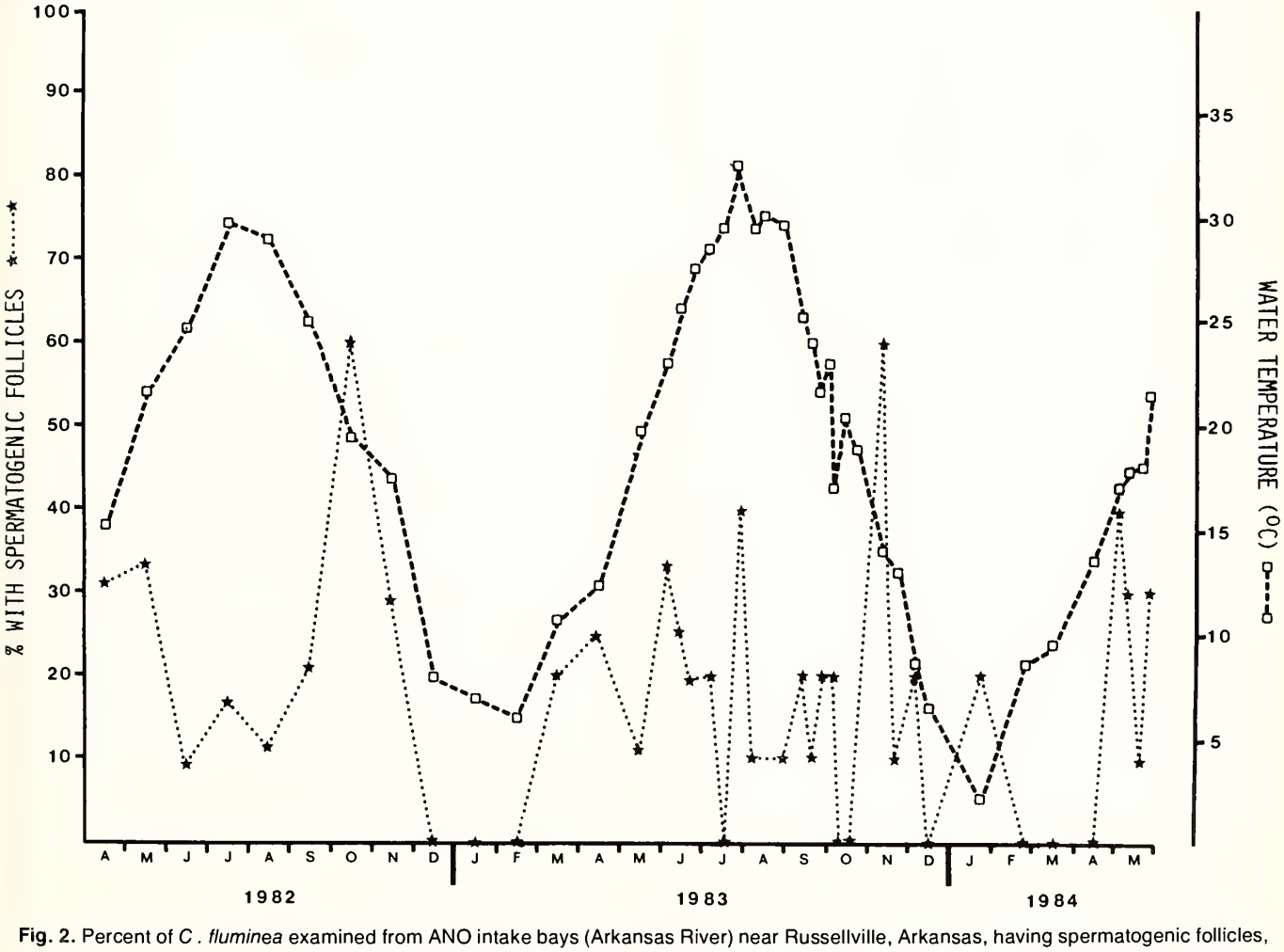

Fig. 2. Percent of *C. fluminea* examined from ANO intake bays (Arkansas River) near Russellville, Arkansas, having spermatogenic follicles, in relation to water temperature. (Note: data point shown for January, 1984 was from shipment which had been held at room temperature for 5 days before dissection.)

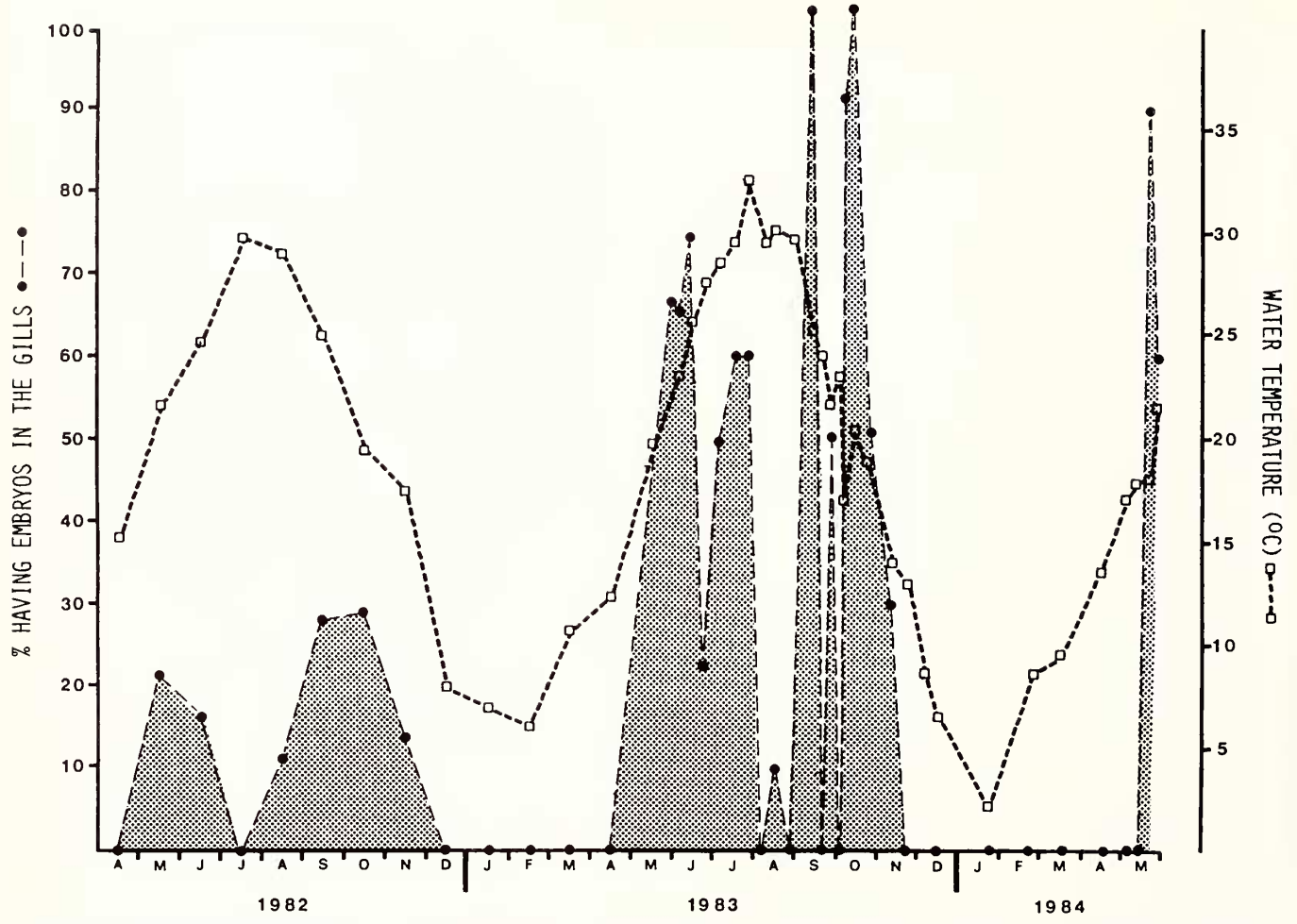

Fig. 3. Percent of *C. fluminea* examined from ANO intake bays (Arkansas River) near Russellville, Arkansas, having embryos in the inner gills, in relation to water temperature.

tissue dissections of the visceral mass. (3) Finally, AP&L personnel have noted that the greatest likelihood of a "clam clog" at ANO in Russellville, has regularly been during the fall.

FERTILIZATION

Earlier studies (Kraemer 1978, 1979, 1984; Kennedy *et al.*, in press; Kraemer, *et al.*, in press) had adduced that *C. fluminea* carries out both self fertilization and cross fertilization. Cross fertilization apparently occurs when spheres of mature sperm make their way out of the gonopores, which are paired and located on either side of the posterior, dorsolateral aspect of the visceral mass (Kraemer, 1978), where the gonopores open into the subrabranchial cavity. Sperm then may be carried to the exterior via the excurrent siphon of the clam and through the water to the siphons of neighboring clams. In this study we repeatedly observed that sperm cells separate from the spheres in the dilute external environment. Sperm thus appear to be transmitted as individual cells. A similar phenomenon regarding the separation of sperm from sperm "morulae" has recently been analyzed in the polychaete, *Arenicola sp.* (Bentley, 1985).

Self fertilization apparently occurs late in the fall reproductive pulse (late September and October in Arkansas) and involves regions of the "follicular ganglia" (Kraemer 1978, 1980, 1984, in press) in areas of contiguity between oogenic and spermatogenic follicles. Serial sections reveal the presence of many embryos there, most being in blastula or gastrula stages. Identification of intrafollicular embryos by means of fresh tissue dissection (as noted in Materials & Methods) showed these also to be usually blastula or gastrula stages.

In this study it was possible to visualize the jelly coat of the oocyte with SEM, along with the yolky cytoplasm and conspicuous nucleus (Fig. 4c,d). Relative size of the oocyte and mature, biflagellate sperm are shown in Fig. 4c,d, though the actual process of sperm penetration was not encountered in our freeze-cracked, SEM preparations. It is possible to identify fertilized eggs in fresh tissue dissections, as they manifest (1) a clearly visible depression in the egg cytoplams, the apparent penetration site (Fig. 5a); and (2) a fertilization membrane and evident loss of the oocyte's gelatinous coat (Fig. 5).

CLEAVAGE, BLASTULA FORMATION

Cleavage in the *C. fluminea* embryo produces coeloblastula comprised of a spherical mass of yolk-laden blastomeres of similar size, which enclose a central cavity. It has been possible to visualize blastulae in serial sections of the visceral mass (evidently a consequence of self-fertilization as noted above). Blastulae have also been dissected from gravid gill chambers (Fig. 6a,b). Blastulae typically measure 175 μm in diameter, and form within 24 hours after fertilization if the water temperature is suitable.

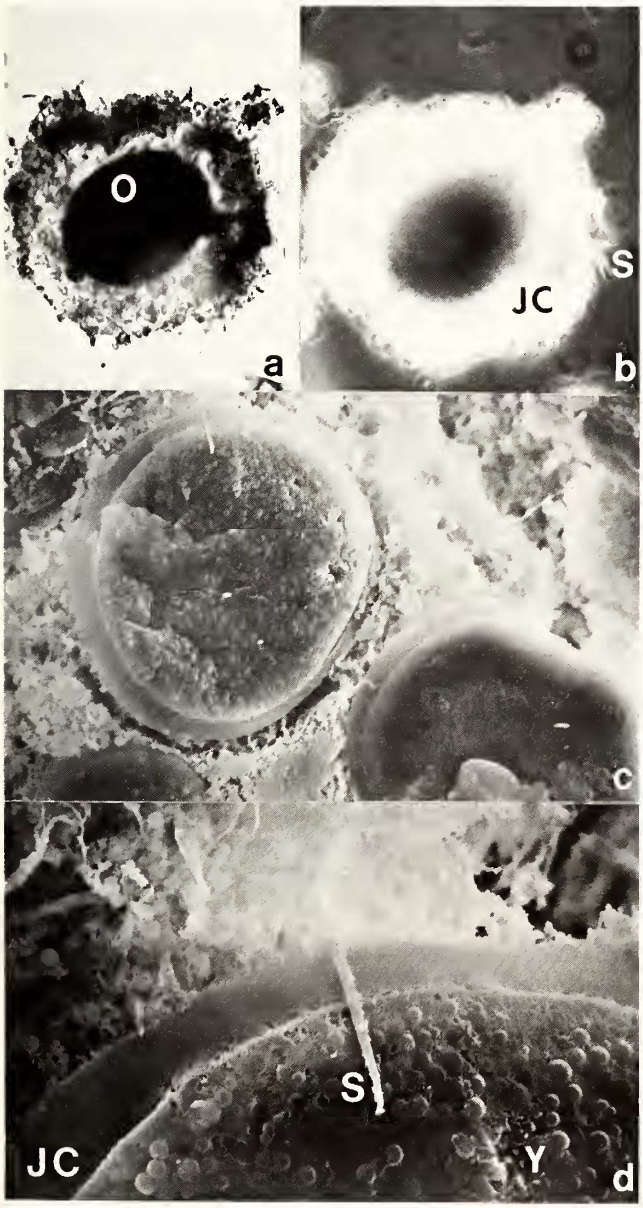

Fig. 4 a,b. Photomicrographs of mature ovum of *Corbicula fluminea* surrounded by a jelly coat containing many embedded sperm. (a) Horizontal field width = 350 μm. (b) Photographed with phase contrast microscopy. Horizontal field width = 400 μm. **c,d.** SEM micrographs of ova, showing freeze-cracked surface of yolky cytoplasm and jelly coat. (c) Horizontal field width = 235 μm. (d) During preparation of the tissue, a mature sperm cell came to lie on the surface of the ovum edge. Horizontal field width = 57 μm. JC, jelly coat; O, ovum; S, sperm; Y, yolk.

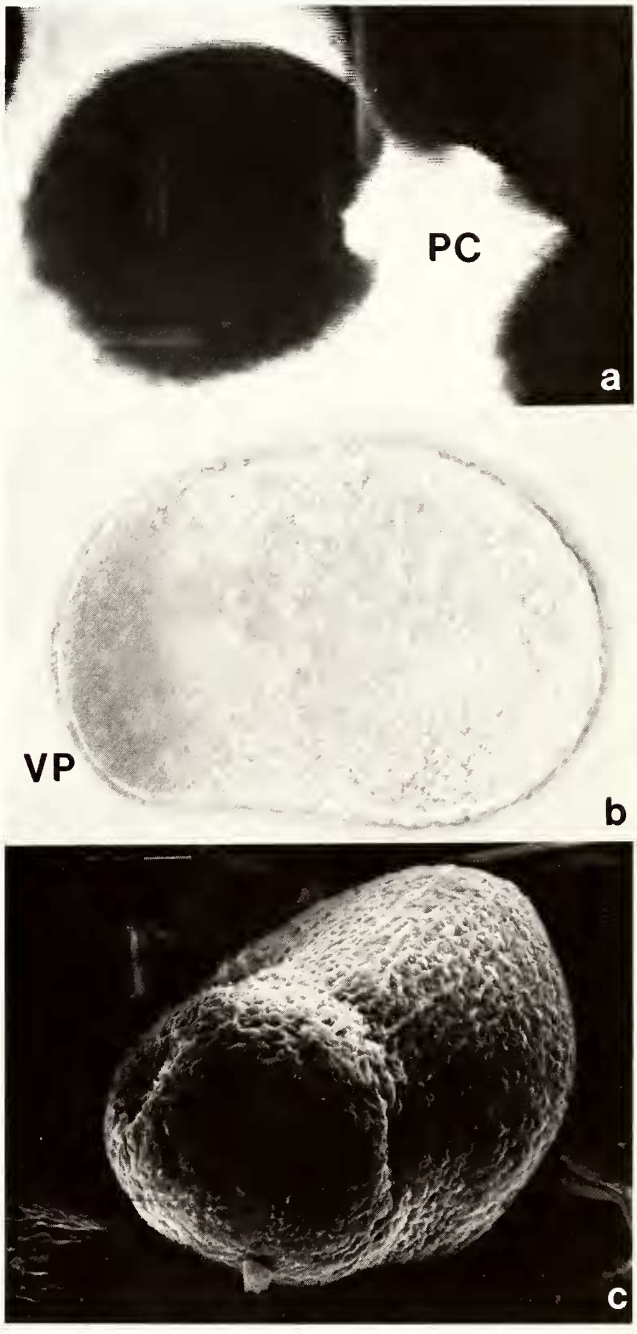

Fig. 5 a-c. Fertilized eggs of *Corbicula fluminea*. (a) Photomicrograph of fertilized eggs, from a videotape, showing evident fertilization or penetration cones, PC. Horizontal field width = 320 μm. (b) Photomicrograph of a fertilized egg as it appears in reflected light, showing more dense aggregation of yolk at the vegetal pole, VP. Horizontal field width = 240 μm. (c) SEM of fertilized egg. Horizontal field width = 230 μm.

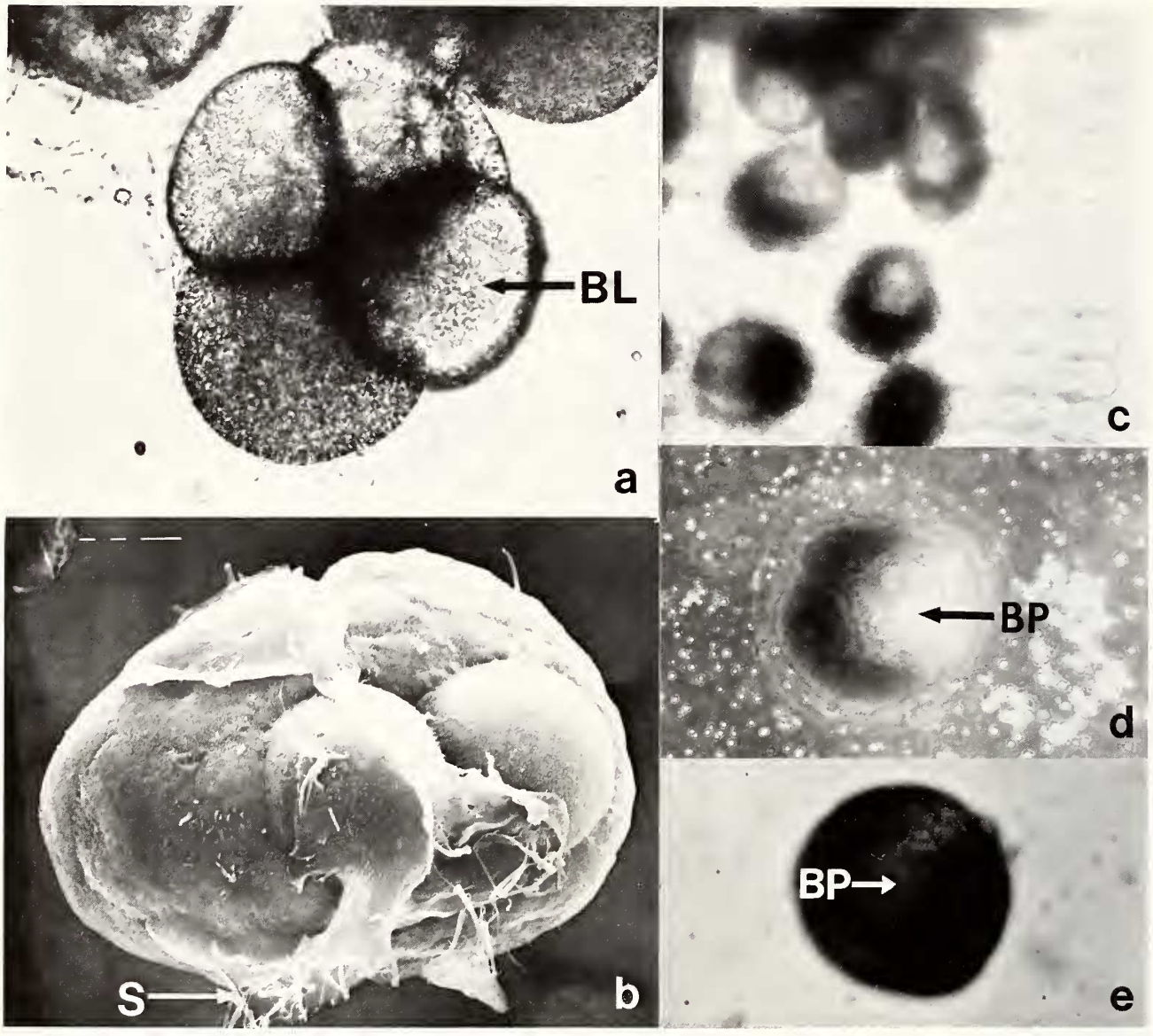

Fig. 6 a-e. Cleavage, blastula and gastrula stages of *Corbicula fluminea*. (a) photomicrograph of cleavage, showing blastomeres, BL. Horizontal field width = 260 μm. (b) SEM of blastula. Horizontal field width = 160 μm. (c) Photomicrograph of gastrulae in marsupial gill. Horizontal field width = 700 μm. (d) Photomicrograph of gastrula, phase contrast. Horizontal field width = 285 μm. (e) Photomicrograph of gastrula. Horizontal field width = 285 μm. BP, blastopore.

GASTRULA FORMATION

Following blastulation, cell proliferation and cell migration produce a gastrula which is bluntly cone-shaped. A large blastopore provides the vegetal pole of the gastrula with an almost flared appearance (Fig. 6c,d,e). Gastrulae appear about 30 hours after fertilization and measure 175-180 μm in diameter.

DEVELOPMENT OF THE TROCHOPHORE

In dissections of fresh tissue of *C. fluminea*, trochophore larvae were frequently seen in the inner, marsupial gills. They could be visualized with SEM, packed into the gill chambers and measuring about 180 μm long. With light microscopy we observed the living trochophores at length as they made their way out of the gill chambers (when artificially freed from gill membranes) and either drifted passively or swam actively there (Fig. 7). Invariably the apical cilia (Scheitelorgan) were "anterior" as the trochophore swam vertically, horizontally or occasionally in a circular path along with other trochophores. When thus observed, the apical cilia of the trochophore are quite mobile and will bend or momentarily retract as the trochophore comes in contact with other larvae or with predators.

For marine bivalves there appears to be some discrepancy in the literature as to which larval stage is a trochophore and which is a veliger. Kume and Dan note (1968, p. 500):

> "No drastic change in body form is involved in the shift from trochophore stage to veliger stage and the boundary drawn between the two stages varies from one investigator to another. The present description (of Kume and Dan) will treat the period lasting until the larval shell becomes prominent as the trochophore stage."

Galtsoff (1965) reports that the marine bivalve, *Crassostrea virginica*, developes distinct valves while still a trochophore, and before the velum appears. In our present study of *C. fluminea*, the trochophores appeared radially symmetrical with light microscopy. With SEM, however, we were able to discern initial development of shell valves during the latter part of the trochophore stage. Like Kume and Dan (1968) and like Waller (1981) for *Ostrea edulis*, we wish to designate the trochophore stage of *C. fluminea* as that period in the development of the clam when it retains an ovoid shape and, with light microscopy, shows no distinct shell valves and no velum.

In the course of this study, trochophores were rarely found in the water surrounding the clams. On a number of occasions it was observed that trochophores released into the water would swell in evident osmotic response. Concomitant behavioral change to a wobbly, attenuated swimming movement, impelled us to conclude that the trochophore larva of *C. fluminea* is not well suited to a free-living, freshwater habitat. This conclusion affirmed that earlier contention (Kraemer, 1979a) that the trochophore does not appear to be the usual distributional larval stage for the species. Just why *C. fluminea* persists in producing a trochophore, a larval stage which *is* the distributional stage for many marine species, will be considered below.

VELIGER LARVA

Observations made throughout several seasons of developmental sequences produced evidence in our study that veliger larvae are regularly developed by *C. fluminea* within the marsupial gills of the parent. Transformation of the trochophore into a veliger is indicated by the development of an asymmetrical profile of the trochophore, when viewed with the light microscope. An asymmetrical aspect results from the growth of the primordia of the shell valves which saddle one side of the "posterior" end of the embryo. Concurrently growth and thickening of the ciliated velum occurs, as it develops from a bilobed outgrowth of the prototroch, just posterior to the Scheitelorgan. The Scheitelorgan persists, and is still tactile and retractile; but the veliger as a whole moves only sluggishly. The velum continues to protrude through the growing shell valves, and indeed cannot be completely withdrawn. Veligers are fully formed from trochophores in about 24-48 hours. Typical length of the veliger measures 190-250 μm (Fig. 8a,b,c).

When veligers were exposed during this study to water surrounding the clams, tissues of the veligers often became

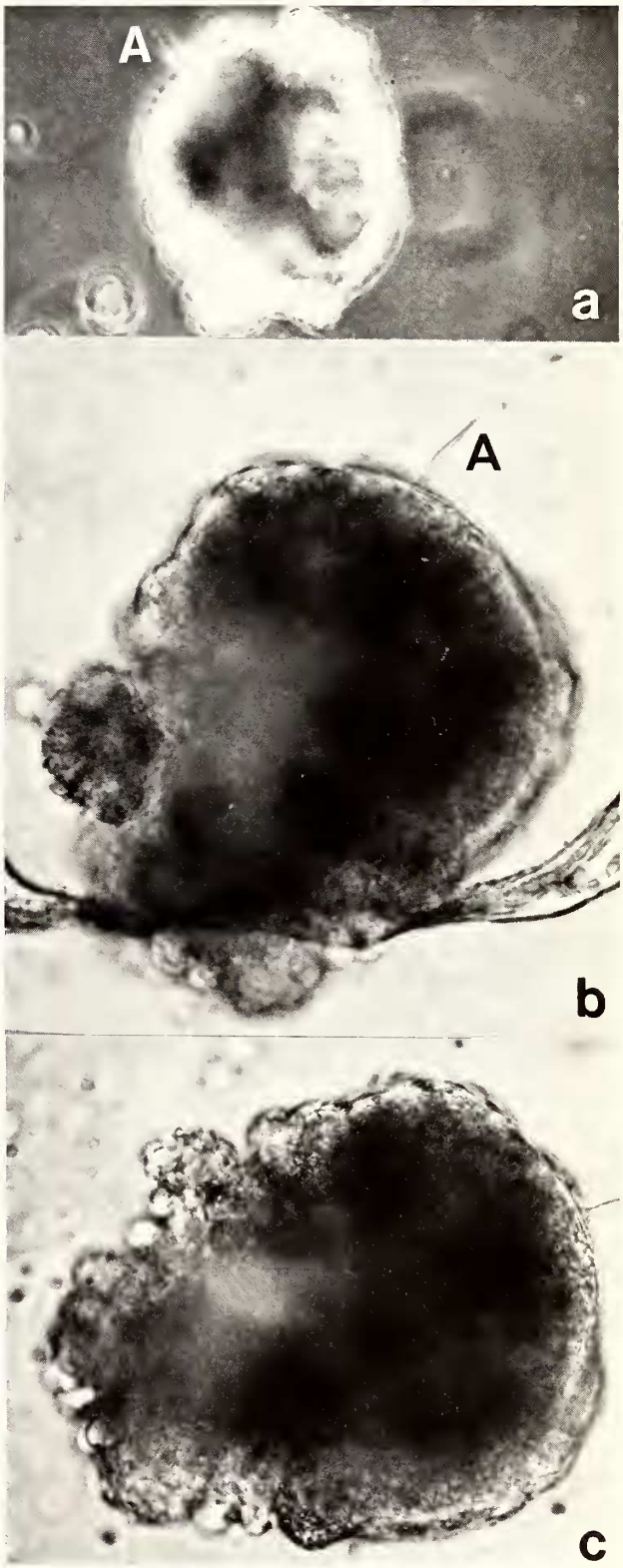

Fig. 7 a-c. Photomicrographs of trochophores of *Corbicula fluminea* from gravid gill. (a) Trochophore photographed with phase contrast. Horizontal field width = 420 μm. (b), (c) Horizontal field width = 205 μm. A, apical cilia (Scheitel-organ).

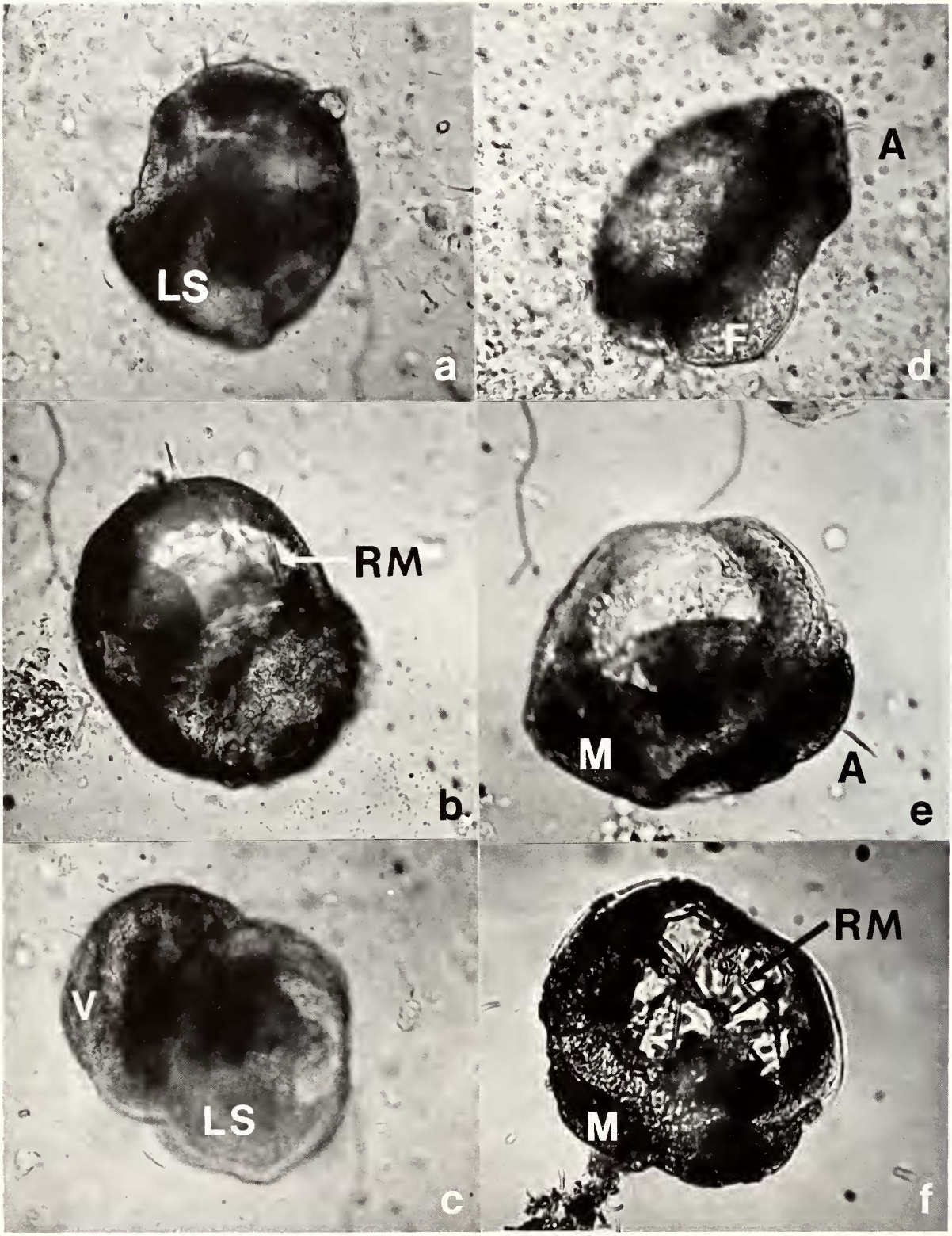

Fig. 8 a-f. Photomicrographs of veligers and early pediveligers of *Corbicula fluminea*. (a), (b), (c) veligers. (b) especially, shows swollen aspect of a veliger in osmotic distress after being exposed to river water. (d), (e), (f) pediveligers. (d) velum and foot extended, mantle retracted. (e) velum extended; mantle extended in posterior region. (f) velum extended; obscuring extended foot. A, apical cilia; F, foot, LS, larval shell valves; RM, velar retractor muscle; V, velum. Horizontal field width = 255 μm.

swollen (Fig. 8a,b). The veliger stage, like trochophore, seems not well suited to a free-living habit in fresh water. It therefore appears unlikely to us that the veliger is a distributional stage for *C. fluminea*.

PEDIVELIGER LARVA

Lengthy observations of living embryos also produced clear evidence of the presence of a pediveliger stage in the ontogeny of *C. fluminea*. The Scheitelorgan persists in this stage. Distinctive characteristics of the developing pediveliger include: (1) The juvenile foot develops immediately posterior to the velum. The enhanced magnification-resolution or our videomicroscopy apparatus enabled us to distinguish the incipient foot from the velum, since the former is a translucent, ciliated, triangular projection of tissue adjacent and posterior to the opaque velum. (2) Larval shell valves grow so that the velum may almost be retracted between them. Subsequent growth of the valves allows complete retraction of the velum, late in the pediveliger stage. The pediveliger stage lasts about 3-5 days. The fully formed pediveliger has straight-hinged valves which measure about 230 μm in length (Fig. 8d,e,f).

While the opaque-appearing velum is still clearly evident and "marked" by the persistent Scheitelorgan, there is another opaque area present which encircles the periphery of the developing animal inside the valves. The latter opaque tissue becomes most evident near the end of the pediveliger stage. The tissue is extensive and bilateral and is especially apparent in the posterior region of the young clam in the early juvenile stage. That it surrounds the differentiating rectum in the region where the siphons and siphonal pocket will eventually develop, is evident from the fact that we have seen fecal material discharged from between the lobes of opaque tissue there (Fig. 9d).

SHEDDING OF THE VELUM AND TRANSITION TO STRAIGHT-HINGED JUVENILE

The veliger shell valves broaden and lengthen during their growth in the pediveliger stage. The velum of the well developed pediveliger is readily withdrawn between the valves by means of the fully differentiated velar retractor muscles (Fig. 8f).

During this study late pediveligers (under pressure from a coverslip) were often observed to extend the velum and to adduct their valves repeatedly or to sustain valve adduction while the velum remained extended. Such behaviors frequently resulted in the casting off of the velum (Fig. 9a). However, there were many observations of the spontaneous shedding of the velum (Fig. 9b), which enabled us to recognize a characteristic, smooth, very distinct convex curve in the distal edge of the visceral mass just at the site where the velum was lost. This "abscission site" can be recognized readily even though the shed velar tissues are gone (Fig. 9c).

It seem inappropriate to use the term, "metamorphosis," for events accompanying shedding of the velum in *C. fluminea*. Nothing comparable to the extensive loss of other larval structures, which coincides with loss of the velum in marine bivalves such as *Crassostrea virginica*, takes place when the velum is shed by *C. fluminea*. Because the velum is removed while the shell valves are still straight-hinged, we have designated the developmental stage in *C. fluminea* which follows velum removal, the straight-hinged juvenile. We are aware that there is no comparable stage in the development of marine bivalves, which retain their velum well through the umbonal stage. Implications of the foregoing events as they relate to the heterochronous development which we have clearly discerned in *C. fluminea*, will be discussed below.

In the transition from pediveliger to the early, straight-hinged juvenile stage, growth of the foot is accompanied by visible change in its form and function, from pointed and inactive to long, sock-shaped and highly mobile. The early juvenile foot is very large and constitutes about one-third of the volume of the animal housed within the valves. There is no significant change in valve dimensions from late pediveliger through early juvenile stages, approximately 230 μm.

THE STRAIGHT-HINGED JUVENILES, EARLY AND LATE

By far the most active developmental stages of *C. fluminea* are the early and late juvenile, straight-hinged stages. With the help of videomicroscopy (described above) it is possible to observe details in the transition of the young clam from its early to late, straight-hinged stages: (1) The gills develop from simple loops attached to the mantle and to the differentiating visceral mass, and then become double loops covered with large, multiple cilia or cirri (Fig. 10c,d,e). The latter, beating like paddles, can be seen sorting particles in the gills. (2) Opaque tissues seen earlier at the posterior and ventral margins of the mantle, gradually disappear. That the former is yolk material we have verified with SEM. (3) The heart develops from a single pulsing chamber to a beating ventricle attached to two membranous auricles. (4) Development of the valve and foot musculature can be followed, as the pedal retractors and protractors which are initially located near the tip of the growing foot, extend dorsally to near the top of the visceral mass at the hinge. (5) The posterior part of the gut and rectum differentiate and become functional, and the production of fecal material can be seen well before the siphons have differentiated. (6) The anterior part of the gut and the style sac differentiate and can be seen to swirl one-celled algae down into the ciliated vortex of the stomach. Some juveniles removed from the marsupial gills had green algae as gut contents, thus indicating that the juvenile clams can feed while they are still in the marsupial gills of the parent. (7) Development of the mantle and the pallial musculature can be monitored and seen to function in the sequence of foot withdrawal, valve adduction, and pallial closure in the juvenile clams. (8) Differentiation of the pedal ganglion and of the statocysts can be clearly observed. With videomicroscopy the statocysts are distinctly seen to be paired and conjoined in the midline. Until the present study, the only other visible evidence of the statocyst organization in *C. fluminea* was from the study of microscopic, serial cross sections by Kraemer (1978a).

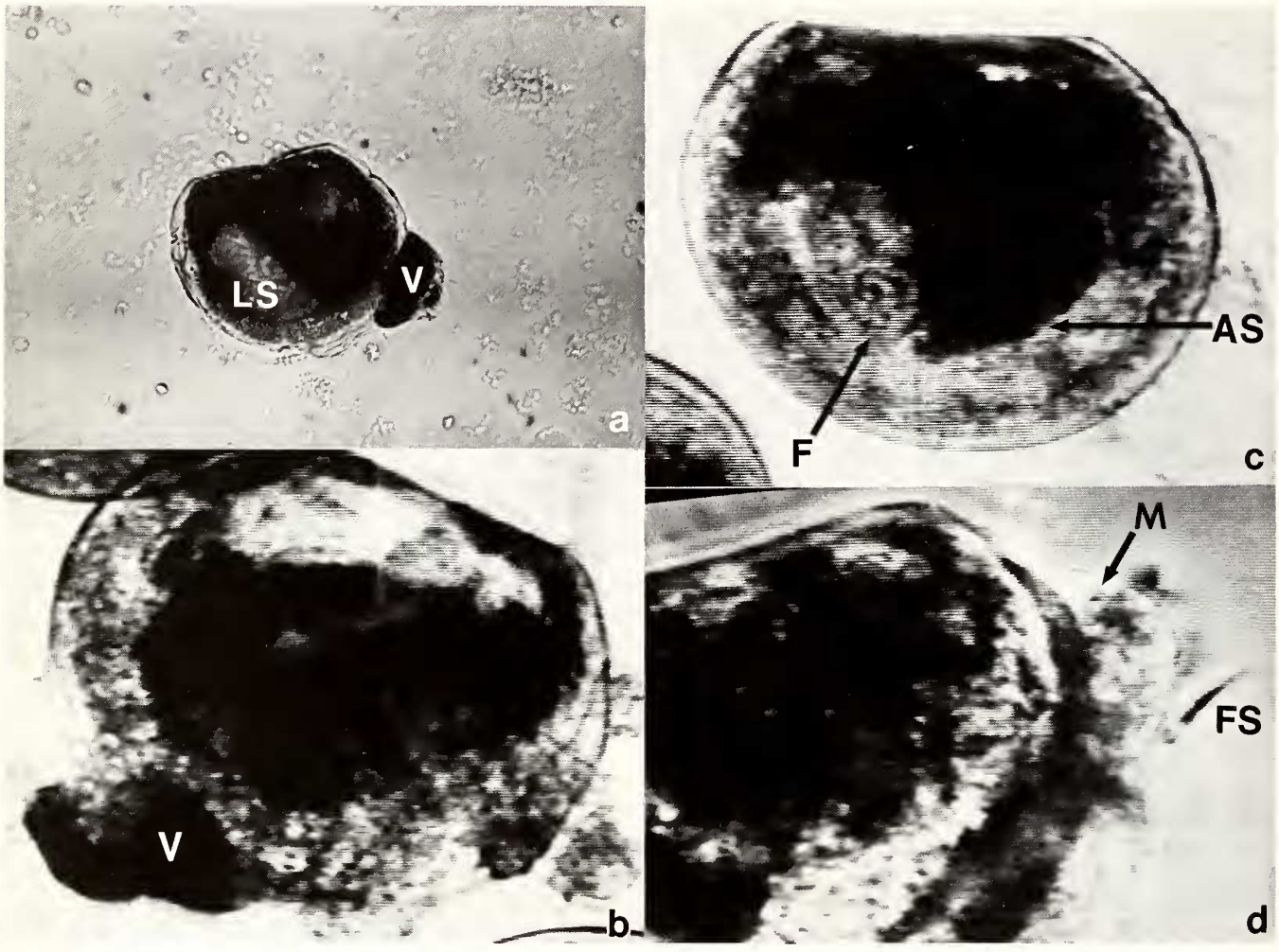

Fig. 9 a-d. Photomicrographs of *Corbicula fluminea* during transition from late pediveliger to early juvenile stages. (a) Late pediveliger shown "casting off" the velum, after repeated adduction of valves was induced by pressure of coverslip on the embryo. Horizontal field width = 570 μm. (b) Late pediveliger shown spontaneously casting off velum. Micrograph from videotape. Horizontal field width = 250 μm. (c) Early juvenile, immediately following casting off of velum, showing "abscission" site from which velum was recently detached. Micrograph from videotape. Horizontal field width = 260 μm. (d) Photomicrograph from videotape of young juvenile showing fecal strand emerging from between distended mantle lobes. Although siphons have not yet formed, posterior region of gut has differentiated and is functioning. Horizontal field width = 230 μm. AS, abscission site; F, foot; FS, fecal strand; LS, larval shell; M, mantle; V, velum.

What has not been appreciated until the present study, is the fact that the statocysts are large (approaching adult size at 15 μm) and well differentiated in the juvenile clams. With videomicroscopy the statocysts can be observed during foot movements. In the early juvenile, the statocysts are located in the distal half of the foot, (Fig. 9c) and in the later juvenile the statocysts are found in the proximal third of the foot (Fig. 10b,c,d). It is apparent that the change in position of the statocysts is due to progressive lengthening and differentiation of the foot. High-power videomicroscopy allowed us to note that the statoliths in both statocysts are also differentiated and move continuously as the juvenile clam's foot moves. The statocysts of *C. fluminea* are much implicated in the movements of the juvenile foot.

In the course of the present study, SEM micrographs of the foot of the juvenile clam revealed a series of 10-12 membranous laminae which comprise the outer surface of the foot (Kraemer, 1984). Examination of serial sections had shown the existence of a "segmental" array of horizontal strands of connective tissue and muscle fibers repeated in the interior of the foot from the distal to the proximal portion of the foot. Videomicroscopy enabled us to see the arrangement of the horizontal "ligaments" in the foot and to appreciate the structural/functional basis for the very active, telescoping movements of the juvenile foot. The locomotor behavior of the juvenile clam does not resemble that of the adult. The juvenile readily swings its foot forward or backward, from side to side in a circular movement, or uses the foot to somersault the rest of its body. The shell valves gape widely, and along with the pallium assist the juvenile clam in cling-

ing to bits of detritus, or in floating in the water column, once it is shed. Some workers have reported finding adult clams floating, alive, in the water column (Bob West, personal communication). Prezant and Chalermwat (1985) have evidence to indicate that the adults may drift on mucus strands in the water column and thereby distribute themselves through the benthos. Our studies on the early developmental stages indicate that the straight-hinged juveniles may also ride water currents to new benthic settlement sites.

Viewing the foregoing developmental stages together, we note that there is substantive change of form in the ontogeny of *C. fluminea* between the trochophore stage and the pediveliger stage, when bilateral symmetry is imposed on the larva and the apical organ becomes the anterior end of the young clam. Changes occurring during the development of a pediveliger to an early, straight-hinged juvenile stage involve differentiation of the foot, pedal ganglion, statocysts and gills, and simple casting off of the velum. Little growth occurs between the pediveliger and early, straight-hinged juvenile stage. Further differentiation *and* shell valve growth (to about 240 μm) characterize the development of the later straight-hinged juvenile stage, where it is lodged in the marsupial gill and after it is shed into the environment (Summary diagram, Fig. 11).

RELEASE OF LARVAE FROM PARENTAL GILLS INTO THE ENVIRONMENT

As mentioned above, a difficulty encountered early in the present study was that shipment of clams in river water resulted in premature shedding of embryos from the parental gills. Another observation made repeatedly was that trochophores and veligers, when exposed to ambient water, would often swell and exhibit stressed behavior. The trochophores and veligers of *C. fluminea* are probably not typically used by these freshwater clams for dispersal of their populations. Early pediveligers, furthermore, exhibit only limited mobility and little coordinated movement. When the larval shell valves are just beginning to develop and the velum is not yet thereby hampered in its movement, the young pediveliger may exhibit some coordinated swimming behavior. As the valves grow, they gradually enclose the velum and behavior of the larva becomes increasingly sluggish, as it swims seldom and awkwardly. Late in the development of the pediveliger when the foot has become quite large, the larva is then capable of active pedal locomotion.

From the late pediveliger stage onward, the larvae are capable of migrating through the parental gill tissues and into the siphonal pocket where contractions of the pallial musculature of the parent clam can eject the young clams. While late pediveligers and early juveniles seem, on the basis of this study, to be the usual embryonic stages relased, it is not uncommon for juveniles to be retained within the marsupial gills well into the late straight-hinged juvenile stage. Water temperature and dissolved oxygen are two significant factors which evidently alter timing of the stage shed. If, as this study indicates, straight-hinged juveniles are capable of feeding while still in the parental gill cavity, an abundant food uptake by the parent clam may keep these juveniles in the gills.

DEVELOPMENT TO THE UMBONAL JUVENILE AND BYSSAL STAGES

In this study it was possible to rear some juveniles to a size of 500 μm. At 500 μm the shell valves of the young clam have developed distinct umbones (Fig. 12). We saw no umbonal juveniles, however, that had developed a byssus. Since the smallest clams in which one of us (Kraemer, 1976, 1979a) had found a byssus were already about 1mm long, it may be that our inability to raise juvenile clams to that size precluded our witnessing the development of the byssus stage. High mortality occurred in our larval cultures when the young clams reached a valve length of 280-300 μm. This high mortality appeared to be correlated with the disappearance of certain remaining "opaque areas" (described above), especially those in the visceral mass near the gut. From examination of juvenile tissues with SEM (Fig. 10), these areas appear to consist of stored yolk material which disappears as it is utilized by the juvenile clam. Thus even though juveniles were observed to feed, mortality may have been caused by insufficient nutriment as embryonic yolk supplies were exhausted. We also conjecture that the byssus may not form unless other environmental conditions are suitable, including the mechanical stimulus of a perceptible current.

SUMMARY AND DISCUSSION

Earlier studies considered some developmental differences which had become generally evident in *C. fluminea,* the Pisdiidae and for marine bivalves. Kraemer and Lott (1977), Kraemer (1978, 1979a,b) and McMahon (1984) remarked on those features and some of their implications. Morton (1982) made some contrasting observations about Asian populations of *C. fluminea* and *C. fluminalis.* McMahon (1984) also reminded us of the comparatively recent appearance of *C. fluminea* in the fossil record, in contrast to the much more lengthy paleontological record of the Pisidiidae in fresh water.

In this paper we have reported findings from 2½ years of continuous detailed study of the reproductive and developmental status of living populations of *C. fluminea* in the intake bays of Arkansas Nuclear One on the Arkansas River near Russellville, and from other "natural" populations in the region (see Materials and Methods). We have found that rising water temperature in the spring and declining water temperature in the fall is the salient environmental change which predictably stimulates the onset of spermatogenesis in *C. fluminea.* We have found that spermatogenesis in turn "times" the rest of the reproductive and developmental sequence. A continuing puzzle, and one certainly deserving of analytical experimental study, is that the environmental stimulus of *falling* water temperature which precedes the autumnal reproductive phase, appears to be a different stimulus than the *rising* water stimulus preceding the spring pulse (Kraemer and Galloway, 1985). The clam's different

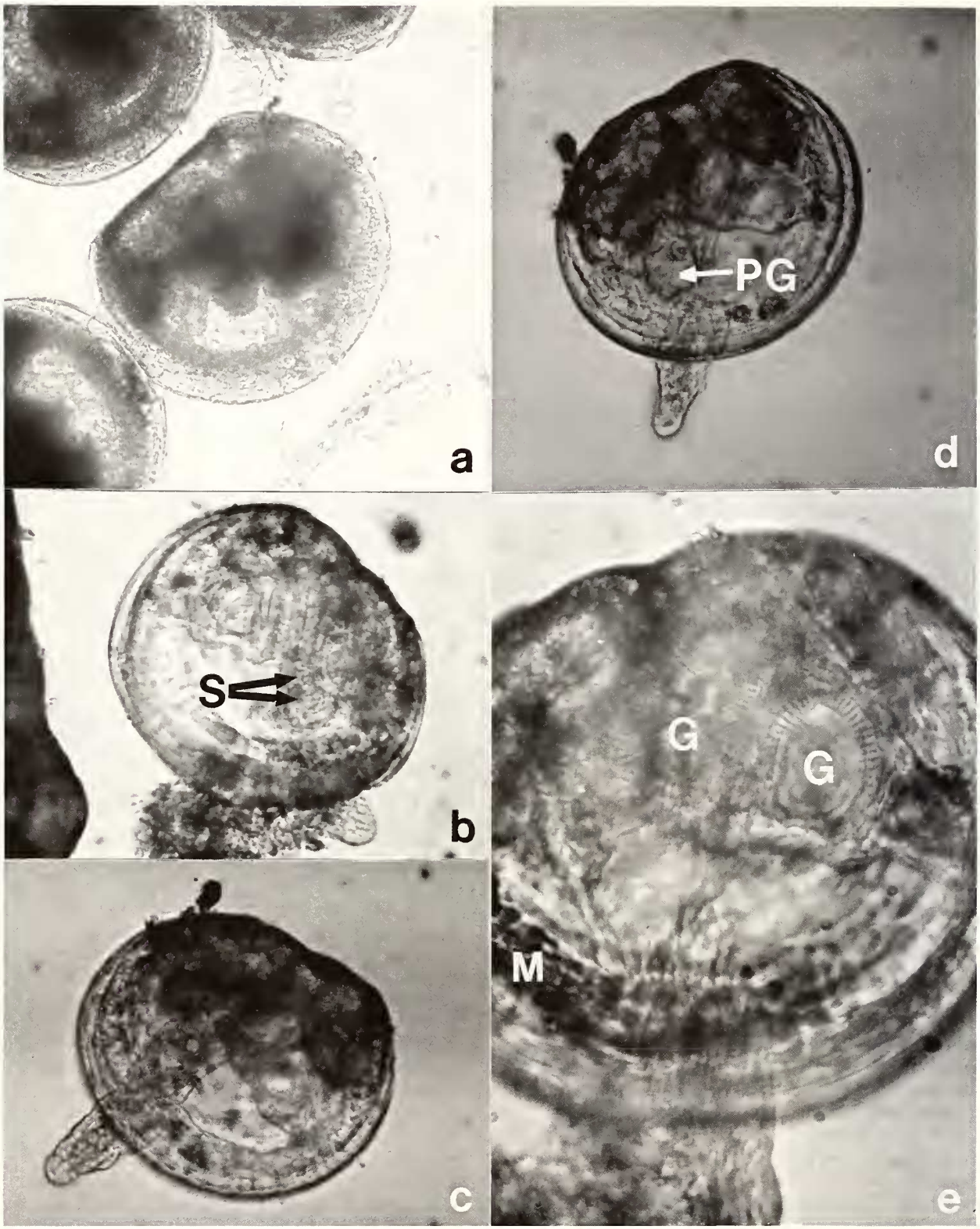

Fig. 10 a-e. Photomicrographs of straight-hinged juveniles. (a) Early, straight-hinged juvenile. (b) Late, straight-hinged juvenile, Oblique view showing, *both* statocysts in the foot. (c), (d) Late, straight-hinged juveniles showing conspicuous, double-looped gills and pedal ganglia. Horizontal field width = 340 μm. (e) Late, straight-hinged juvenile showing double-looped gills with well differentiated cirri. Horizontal field width = 170 μm. G, gill; M, mantle; PG, pedal ganglion; S, statocyst.

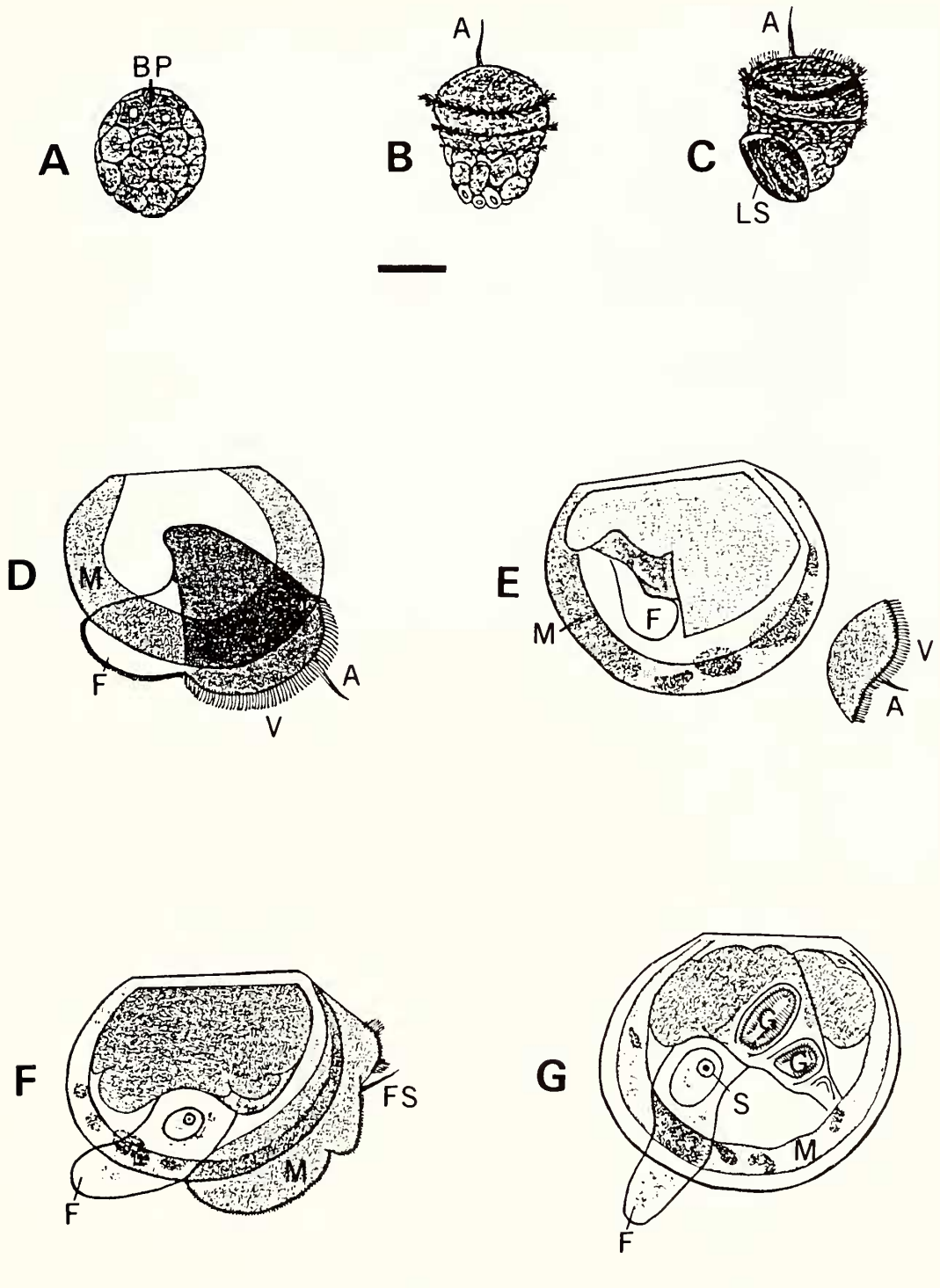

Fig. 11. Summary diagram of developmental stages in *Corbicula fluminea* through the late, straight-hinged juvenile stage. Fertilization, cleavage and blastulation precede A, gastrula stage (shown upside down; B, trochophore; C, veliger; D, pediveliger (anterior end toward right); E, early, straight-hinged juvenile with recently cast off velum, (anterior end toward right); F, early, straight-hinged juvenile (anterior end toward left); G, late, straight-hinged juvenile (anterior end toward left). In this study, embryos were usually shed in the stages from late pediveliger through early and late, straight-hinged juveniles. Two later, post "shedding" stages, the umbonal stage and the byssal stage, are not shown here. Scale bar for a,b,c = 85 μm. Scale bar for d,e,f,g = 55 μm. A, apical cilia; BP, blastopore; F, foot; FS, fecal strand; G, sill; LS, shell; M, mantle; S, statocyst; V, velum; VS, shed velum.

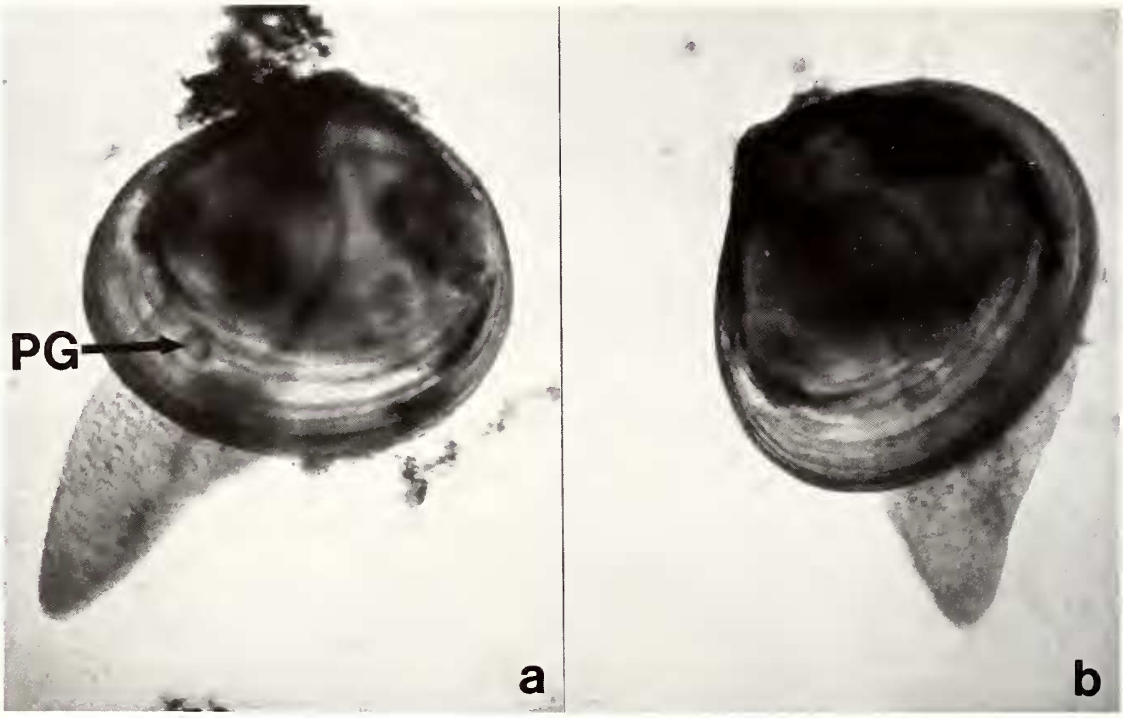

Fig. 12 a,b. Umbonal juveniles of *Corbicula fluminea*. Horizontal field width = 690 μm. PG, pedal ganglion.

reproductive response is surely related to its different metabolic states but may also be affected by the direction and rate of temperature change. Nonetheless, an important result of the present study is the finding that although *C. fluminea* is proto-oogamous, it is *spermatogenesis* that is especially temperature sensitive; and it is spermatogenesis that paces reproductive and developmental processes (see Figs. 2,3). Also, we have found that oogenesis occurs nearly all year long, though it "waxes and wanes" from one season to another.

During the preparation of this paper, we became aware of the ambiguity of the term, "spawning." Spawning has been defined as either the release of gametes or of embryos or young into the environment. *C . fluminea* "spawns" both sperm cells and juveniles. Since some zoologists think of "spawning" as release of gametes only, and others refer to release of young as "spawning" (e.g. Doherty, *et al.,* 1985) we made an effort to avoid confusion, and have eschewed any use of the term.

Though cross fertilization appears to be a typical process for *C. fluminea*, we have found evidence for self fertilization within the gametogenic follicles of the visceral mass, in the fall. Evidence of self-fertilization is summarized in Kraemer, *et al.,* (in press). As noted above, repeated findings were made by Kraemer (1978) on *intrafollicular* embryos in microscopic serial sections of *C. fluminea.* The most parsimonious explanation for these findings is, of course, self-fertilization, i.e., fertilization of mature oöcytes within the oogenic follicles, by mature sperm from the contiguous spermatogenic follicles. Kennedy (1985), though also convinced that self-fertilization occurs in *C. fluminea,* was able to gather only equivocal results from a very painstaking study involving the rearing of *C. fluminea* isolates. The process of self-fertilization obviously requires additional experimental investigation.

We have confirmed that *C. fluminea* regularly produces several sequences of larval stages during each of the two (spring and fall) reproductive seasons. We consistently found the developmental sequence to include: (1) cleavage; (2) blastulae; (3) gastrulae; (4) trochophores; (5) veligers; (6) pediveligers; (7) early straight-hinged juveniles; and (8) late straight-hinged juveniles. (9) Once released into the environment, straight-hinged juveniles eventually grow to a length of 500 μm, in the process differentiating umbonal shell valves; and (10) later producing a byssus when their shell valves approach 1 mm in length. We observed that neither the trochophore stage nor the veliger stage appear well suited to survival in freshwater habitat, but that these stages are typically retained within the gills or mantle cavity surrounding the gills. We continually observed that the young of *C. fluminea* are typically released into the environment in one of the straight-hinged juvenile stages or less often as late pediveligers.

We realize that terms applied to larval stages of marine bivalves both overlap and contrast with terms we have used for ontogenetic events in *C. fluminea.* This is really unavoidable, since our findings clearly show that larval stages in *C. fluminea* actually *do* both overlap and contrast with stages in the development of marine bivalves. Clarification of the embryological terminology used in this paper is offered in Table 1.

Table 1. Comparison of embryonic terms as they apply to an estuarine bivalve, such as *Crassostrea virginica* Gmelin (Galtsoff, 1965) and to *Corbicula fluminea* (Müller) in this paper.

Crassostrea virginica	*Corbicula fluminea*
Fertilization in sea water. Eggs shed into water containing sperm.	Fertilization in marsupial gills or (less often), self-fertilization within gametogenic follicles.
Cleavage.	Cleavage.
Blastula a stereoblastula.	Blastula a coeloblastula.
Gastrula.	Gastrula, cone-shaped with a large blastopore at vegetal pole. 175-180 μm in diameter.
Trochophore, about 60 μm long, with ciliated prototroch as swimming organ. Shell valves prominent with light microscopy.	Trochophore, about 190 μm long, with prototroch and distinct Scheitelorgan, with large, retractile, *motile* apical cilia. Initial development of shell valve observed with SEM.
Veliger, about 70-75 μm long. Velum formed from lateral extensions of prototroch, a *strong swimming organ.*	Veliger, about 190-250 μm long. Velum forms as outgrowths of prototroch around base of Scheitel organ. *Sluggish.* Velar cilia move food particles.
Veliger also called a straight-hinged larva or D-shaped larva. Viewed from dorsal surface, two groups of rectangular teeth are seen on either side of hinge.	Veliger with straight-hinged shell valves. No rectangular teeth lateral to hinge on dorsal surface.
Pediveliger. Larval foot appears.	Pediveliger. Foot appears posterior to velum.
Umbonal veliger, about 300 μm long. Umbones develop on either side of hinge. Well developed velar retractor muscles with striated fibers. Apical organ in center of velum. Gill rudiment present. Pair of statocysts. Pair of larval eyes. Pedal ganglia, pleural ganglia, posterior adductor muscle. Byssal gland opening into mantle at base of foot.	NA
"Metamorphosis." Casting off or disintegration of velum within 48 hours, with "setting" of umbonal veliger. Resorption of foot, degeneration of posterior adductor muscle and larval eyes.	Casting off of velum by straight-hinged pediveliger, to become straight-hinged juvenile, about 230 μm long.
NA	Straight-hinged juvenile is stage typically released from adult into water. Rapid locomotion with juvenile foot. Gills begin to form. Conjoined statocysts, pedal ganglia, esophagus, stomach, intestine, rectum.
NA	Umbonal juvenile stage occurs after 2+ months in subtrate (in laboratory culture) when umbones develop on either side of hinge, and clam is 400-500 μm long.
Byssal stage develops in umbonal veliger, described above.	Byssal stage develops in umbonal juvenile. Byssal thread produced from gland and groove in distal portion of foot.

A number of ontogenetic events in reproduction and development of *C. fluminea* seem anomalous when the species is compared with marine and other freshwater bivalved mollusks. Among these are: (1) *C. fluminea* is clearly proto-oogamous, though its indigenous freshwater relatives, the pill clams and fingernail clams (Pisidiidae), in particular, and marine bivalves in general, are protandrous (Fretter and Graham, 1964; Galtsoff, 1964; Raven, 1966; Heard, 1977; Mackie, 1979; Way et al., 1981). (2) In *C. fluminea* development from cleavage to blastula, gastrula, trochophore, veliger, pediveliger, early and even late straight-hinged juveniles *all* occur within the marsupial gill and branchial mantle cavity. The freshwater Pisidiidae, similarly, retain their developing young within the marsupial gill but for a much longer time, until the young are nearly the size of the parent and have begun sexual differentiation (Heard, 1977). Furthermore, the few young which complete development in the parental gills of Pisidiidae never exhibit a trochophore, veliger or pediveliger stage. Okada (19a,b,c) has evaluated the remarkable suppression of larval stages in the Sphaeriidae. In contrast and as shown above, *C. fluminea* has retained the entire sequence of developmental stages in its freshwater habitat, which is characteristic of many *marine* bivalves. In many species of marine bivalves, of course, the gametes are shed and fertilized in the ocean and all ontogenetic stages are free living there. In some marine bivalve species, such as *Ostrea lurida* and *O. edulis*, eggs are fertilized within the marsupial gills and development proceeds in the mantle cavity, so that well developed larvae are released (Galtsoff, 1964). (3) The *rate* of development is rapid in *C. fluminea*, approaching that of marine bivalves (Galtsoff, 1964), and involves the voluminous turnover of relatively small embryos as several ontogenetic sequelae occur with each seasonal

reproductive pulse. In contrast, as indicated above, direct development of few young in the Pisidiidae is prolonged in the parental gill marsupia. (4) In *C. fluminea* the released juveniles become umbonal and develop a byssus which is used to anchor the young clam to the river bottom. Byssal stages similarly serve to anchor marine bivalves. In the indigenous freshwater Pisidiidae, however, there is a *marsupial* byssal stage, in which the young develop a "placental" byssus which is used merely to attach the juvenile clam to the wall of the marsupial gill chamber (Mackie, 1978). The foregoing, developmental "timing" differences are summarized in Table 2.

We note that ontogeny of the introduced Asian clam, *Corbicula fluminea*, when compared with the ontogeny of its indigenous freshwater relatives, the corbiculacean pill clams and fingernail clams (Pisidiidae) *and* with the ontogeny of many marine bivalves, exhibits significant developmental "timing" differences. *C. fluminea* is obviously not nearly so well adapted to a "natural" freshwater habitat as are the Pisidiidae.

Ontogenetic events in *C. fluminea* are still very similar to those of marine bivalves, which normally develop *free living* trochophores and veligers. Heterochrony as "phyletic change in the onset or timing of development . . . either accelerated or retarded relative to the . . . rate of development of the same feature in an ancestor's ontogeny," that is in the sense in which De Beer used it (Gould, 1977), — seems evident in the larval development and larval ecology of *C. fluminea*. Since a sexually mature clam can release thousands of well-differentiated, straight-hinged juveniles during a reproductive season (McMahon, 1984) directly into the environment, it would obviously require few such clams to establish a local population quickly. The peculiar development of *C. fluminea* contrasts with that of marine bivalves, which typically rely on planktonic larvae for their distribution. Embryogenesis in *C. fluminea* also contrasts strongly with that of the freshwater Pisidiidae (pill clams and fingernail clams) which produce a very few, large, well-developed young per season. Also, the freshwater Unionidae (Mussels) which individually produce thousands of glochidia larvae that typically

Table 2. Evident heterochrony in the comparative ontogeny of some Corbiculacea: *Corbicula fluminea, Pisidium* and *Sphaerium* Gametogenesis to pediveliger stage.

ONTOGENETIC EVENT*	TIME COURSE OF EVENT	
	Corbicula fluminea	*Pisidium, Musculium*
Oogenesis	precedes spermatogenesis; occurs throughout the year	follows spermatogenesis
Spermatogenesis	follows oogenesis; seasonal, temperature sensitive; "times" reproduction	precedes oogenesis
Sperm	biflagellate	uniflagellate
Cleavage, blastulation	within 24 hours., usually in marsupial gill	——
Gastrulation	usually within 12-24 hrs., in marsupial gill	——
Trochophore	24-48 hrs., in marsupial gill	suppressed
Veliger	24-48 hrs., in marsupial gill	suppressed
Pediveliger	48-96 hrs., in marsupial gill, usually	suppressed
Early juvenile (straight hinge)	24-48 hrs.	? (within marsupial gill)
Late juvenile (straight hinge)	2+ months	? (within marsupial gill)
Shedding* (release from gill)	often as late pediveliger or later	much later in development
Umbonal juvenile	occurs long after shedding when juvenile has attained length of 500+ μm	occurs within marsupial gill
Byssus formation	occurs still longer after shedding when umbonal juvenile attains a length of 1+ μm	*precedes* 1st juvenile stage. occurs within marsupial gill, before shedding, as "placental" byssus.
Gametogenesis	occurs after shedding, after byssus formation, etc.	may occur in "juveniles" within marsupial gill.

*Time course of development, from fertilization (zygote formation) to shedding of late pediveliger or straight-hinged juveniles from marsupial gills of *C. fluminea* is approximately 6-12 days, normally. While in some instances embryos may be retained into late, straight-hinged juvenile stage within marsupial gills, some embryos may be released as early as 5 days after fertilization when the embryos are still pediveligers. Rarely, fertilized eggs, trochophores, or veligers are shed. Trochophores and veligers may exhibit osmotic stress.

require a parasitic period on a specific host fish, contrast with the rapid direct development of juveniles in *C. fluminea*. The ontogeny of *C. fluminea* seems admirably well suited to survival and propagation in the stressed, unstable habitat of many rivers in the U.S. today (Kraemer, 1979; McMahon, 1984). In many ways intermediate between the ontogeny of marine bivalves and of the freshwater Pisidiidae, and neither marine-like nor freshwater-like, the embryology of *C. fluminea* seems well matched to the calamitous events which attend freshwater "ecological crunch" (Wiens, 1977). The heterochronic, ontogenetic "timing" of *C. fluminea* seems very likely to be the main key to its present "success" in U.S. rivers.

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